

Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony

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SUMMARY

Vertebrate Hox genes are essential for the proper organization of the body plan during development. Inactivation of these genes usually leads to important alterations, or transformations, in the identities of the affected developing structures. Hox genes are activated in a progressive temporal sequence which is colinear with the position of these genes on their respective complexes, so that 'anterior' genes are activated earlier than 'posterior' ones (temporal colinearity). Here, an hypothesis is considered in which the correct timing of activation of this gene family is necessary in order to properly establish the various expression domains. Slight modifications in the respective times of gene activation (heterochronies) may shift expression domains along the rostrocaudal axis and thus induce concurrent changes in morphologies. It is further argued that temporal colinearity only occurs in cells with high mitotic rates, which results in a strong linkage between patterning and growth control and makes the patterning process unidirectional, from anterior, proximal

and early, to posterior, distal and late, a model referred to as the 'Einbahnstrasse'. While the nature of the mechanism(s) behind temporal and spatial colinearities is unknown, it is proposed that such a mechanism relies on meta-*cis* interactions, that is it may necessitate gene contiguity. Such a mechanism would be based on DNA-specific, rather than gene-specific, features such as chromatin configurations or DNA replication. The existence of such a meta-*cis* mechanism would explain the extraordinary conservation of this genetic system during evolution as its basic properties would be linked to that of the genetic material itself. Consequently, it is hypothesized that, in vertebrates, the resistance of this mechanism to evolutionary variations may be the reason for the existence of a short developmental window of morphological invariance (the phylotypic progression).

Key words: Hox genes, vertebrate evolution, gene activation, expression domains

INTRODUCTION

In vertebrates, 38 genes contain a homeobox sequence related to that of the *Drosophila Antp* gene and encode sequence-specific DNA binding transcription factors (reviewed by McGinnis and Krumlauf, 1992). These genes are clustered in four complexes and are members of the so-called Hox gene family. Each complex contains 9-11 genes, regularly spaced over about 200 kb, and transcribed from the same DNA strand. Sequence analyses have revealed that vertebrate HOX complexes can be aligned with the *Drosophila* Antennapedia and Bithorax complexes (ANT-C; BX-C) of homeotic genes when these latter two clusters are conceptually linked together, indicating a common phylogenetic origin of the complexes (Duboule and Dollé, 1989; Graham et al., 1989). This led to the conclusion that, during evolution, an ancestral complex (Hox/HOM-C) was split, in Diptera, and also amplified along the lineage leading to vertebrates. As a consequence of these cluster duplications, genes located at similar relative positions within the HOX complexes (paralogs) show high sequence

similarities. The relationships between this structural organization and the expression patterns of the Hox genes during fetal development have been extensively studied and reviewed (e.g. Gaunt, 1991). Briefly, genes located at the 3' extremities of the complexes (such as group 1 or 2 genes) are expressed starting at anterior positions within the hindbrain, while genes located at 5' positions (e.g. group 12 or 13 genes) are expressed in progressively more restricted posterior areas (e.g. the genitalia). The same rule can be applied to the expression patterns of Hox genes during limb development (Dollé et al., 1989) and is referred to as 'spatial colinearity' (Gaunt et al., 1988). This type of colinearity was first observed in *Drosophila* by Lewis (1978) and subsequently documented at the molecular level (e.g. Harding et al., 1985).

TEMPORAL COLINEARITY

The anterior-posterior succession in the topography of the Hox gene expression domains may depend on another type of col-

inearity, which is concerned with the time of activation of these series of genes during development. A delay is observed in the appearance of the transcripts encoded by the more 5'-located genes; for example, *Hoxd-13* transcripts appear after those encoded by *Hoxd-10*. The rule is that one can never detect transcripts from a given Hox gene before transcripts are produced by its 3'-located neighbour in the complex. This implies that the physical ordering of the genes along their complexes reflects the temporal sequence of their activation (Dollé et al., 1989; Izpisúa-Belmonte et al., 1991). This phenomenon, referred to as temporal colinearity, is most clearly visible for those genes located at 5' positions but can most likely be extended to more 'anterior' genes, as suggested by work carried out in cultured EC cells (Boncinelli et al., 1991) or in *Xenopus* (Dekker et al., 1992). The onset of activation occurs during early gastrulation (Gaunt et al., 1986; Gaunt, 1987; Deschamps and Wijgerde, 1993), at a stage when the embryo establishes its major body axis, and the process complete, in mice, about 2 days later, at the late tail bud stage (Dollé et al., 1991b).

The key role of vertebrate Hox genes in the proper organization of the body plan is now well documented. Experiments involving either gain of function or loss of function have revealed that these genes are required to build (identify) structures properly, usually within a rostro-caudal window that corresponds to the anterior part of their expression domains (see below). The absence of a given Hox product will often result in the transformation of a structure into a similar, but different, structure from the same anatomical series (e.g. a lumbar into thoracic-like vertebra), transformations that are often explained in terms of homeosis (e.g. LeMouellic et al., 1992; Ramirez-Solis et al., 1993; Rijli et al., 1993). If we consider, as an example, the morphogenesis of the vertebral column, it is thus fair to speculate that the coupling between the anterior-posterior (AP) progression in somite formation and the sequential activation of the Hox genes (in presomitic mesoderm) determines the combination of Hox genes expressed at a given AP level of the sclerotome and, consequently, the shape of the future vertebra. In this view, it is not because more posterior metameres are sequentially produced that posterior Hox genes become successively active, but instead, because subsequent Hox genes are turned on that the newly appearing structures can acquire distinct identities. Hence, any variation in the relative speeds of the two processes would lead to mis-identifications of structures. It is, therefore, of great importance to understand what are the mechanistic and molecular bases of temporal colinearity. I would like to argue first, that temporal colinearity is linked to a particular type of clustered organization and second, that it may be dependent on cellular proliferation.

Circumstantial evidence suggests that proper timing of Hox gene expression may be linked to a highly organized type of clustered organization (Duboule, 1992). In *Drosophila*, an animal that does not appear to use temporal colinearity, the homeotic genes are split in two complexes. Furthermore, in this system, the structural organization of ANT-C is quite different from that observed in both BX-C and the homologous gene complexes in vertebrates, where a higher degree of organization is achieved (Kaufman et al., 1990). In mice, expression patterns resembling the endogenous domains have been obtained with a number of Hox transgenes containing different

amounts of DNA sequence around particular transcription units (e.g. Whitting et al., 1991; Sham et al., 1992; Gérard et al., 1993). However, while these results demonstrate that *cis*-acting elements contribute to the spatiotemporal specificity of expression, the large majority of transgene expression patterns do not exactly reflect the endogenous situations, in particular with respect to the positions of the cranio-caudal boundaries. Therefore, while these data seem to contradict the above hypothesis (that demands that the onset of transcription is determined entirely by the position of a given gene within the complex), they can be reconciled with a model where the complex would be required to refine and coordinate a pre-existing, gene-specific, temporal control.

HOX PATTERNING AND GROWTH CONTROL

The distribution of Hox gene expression domains during development, in particular for the 'posterior' genes, suggests that they are activated in regions of cellular proliferation, as illustrated by the enhanced expression of some *Hoxd* genes in the genital tubercle, in limb buds and in the tail bud (Dollé et al., 1991a,b). Experiments involving manipulation of the mouse or chick limb buds led to the conclusion that the reactivation of *Hox* gene transcription, which always obeys temporal colinearity, can only occur within cells that proliferate (i.e. within those cells that proliferate in order to produce supernumerary structures; Izpisúa-Belmonte et al., 1992; Riddle et al., 1993). Convincing evidence was obtained in the chick wing bud by manipulating both the reactivation of Hox genes and the growth of the additional structure through local release of retinoic acid and subsequent removal of the ectoderm layer. This clearly showed that the process of sequential reactivation was interrupted when proliferation was stopped due to the absence of signaling from the surrounding ectoderm (Izpisúa-Belmonte et al., 1992).

Relationships between growth control and pattern formation is a general feature of epimorphic systems. A mechanistic linkage between the growth of a structure and the processing of its patterning system (like the one postulated here) would help prevent these two aspects of morphogenesis from becoming uncoupled, i.e. prevent growth from occurring faster than patterning or vice-versa. This direct linkage would imply that the HOX complexes act as a mechanism to translate a recurrent process (proliferation) into a linear progression (morphogenesis). Such a relationship is schematized in Fig. 1. While the mechanistic bases of this potential association are unknown, one could imagine that progression in the activation of Hox genes along their complexes would be a function of the rate of proliferation of a given cellular population. In the case of the developing limbs, for example, cells in the progress zone would continually allow further Hox genes to be activated until the time when cells would leave this zone. At this point, the state of activation achieved (e.g. up to *Hoxd-10*) will be maintained in all daughter cells. If a higher rate of proliferation of these cells is resumed, later in development, by producing an extra structure from the anterior margin of the wing bud (e.g. Summerbell, 1981; Cooke and Summerbell, 1981) or in a regeneration blastema (Rose, 1962), the cells will continue to progress on their HOX complexes starting from the point at which they had been stopped (i.e. making *Hoxd-11* available).

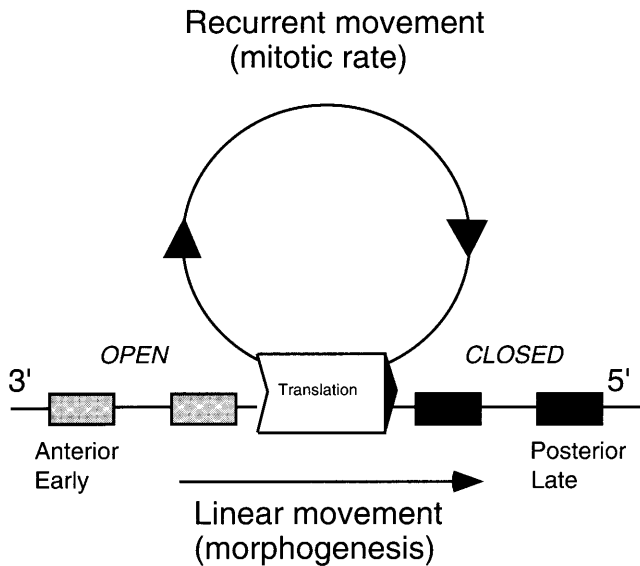


Fig. 1. Schematic illustration of a possible linkage between growth and patterning through the Hox gene complexes. Cells with a high (upper-threshold) mitotic index can 'proceed' along their HOX complexes so that more genes become available (open) for progressively more posterior (distal) patterning. Cells with lower (below-threshold) indexes maintain their state of activation without further opening. In these latter cells, progression can continue if high proliferation is resumed. In this schematic view of one piece of a prototype HOX complex, dark stippled rectangles represent 'closed' genes while light stippled rectangles are 'open' genes. Such open genes may not necessarily be transcribed. The box 'translation' represents the meta-*cis* mechanism referred to in the text. This mechanism may progressively allow more posterior genes to become available in those cells having a high mitotic rate.

This would explain the continuity in patterning observed in the above mentioned experiments, and give a molecular basis to the rule of distal transformation (e.g. Stocum, 1981), the progress zone model (Summerbell et al., 1973) and the acquisition and fixation of 'positional identities' (see Wolpert, 1989).

THE EINBAHNSTRASSE

Such a linkage between growth control and patterning through the HOX complexes would result in the use of series of genes as a system of coordinates; it thus has some traits of the polar coordinate model (French et al., 1976; see also Holder, 1981). However, in marked contrast to this latter model, this system would constrain patterning to be unidirectional and therefore make Hox-dependent intercalation strictly impossible, a proposal previously referred to as the *Einbahnstrasse* (Izpisua-Belmonte and Duboule, 1992). This model states that the patterning system progresses from early to late, from anterior to posterior, from proximal to distal. It also implies that it can stop and start again (at the same position along the complex) but that it is impossible to come back (i.e. to produce 'anterior' or 'proximal' cells with the progeny of 'posterior' or 'distal' ones, respectively). To achieve the latter transitions, cells would need to de-proliferate (i.e. to go backwards in time), which does not appear to be an acceptable possibility. It means

that the process is irreversible and that the designations 'anterior' and 'posterior' are two faces of the same mechanism, acting at different times and always in the same sequence.

At first, such a model seems to be in contradiction to some observations that suggest that, in some systems, posterior body parts can regenerate anterior portions (e.g. Slack, 1980). However, it is not clear whether, in these particular cases, anterior regeneration does require posterior cells (as defined by the expression of posterior Hox genes) to become anterior. It is conceivable that those cells engaged in such 'anterior' regeneration did not previously express any Hox genes or, alternatively, that one of the first responses to experimental injury is to erase any Hox expression, thus allowing cells to progress again from anterior to posterior.

To some extent, the hypothesized relationship between cell proliferation and Hox genes can also be assessed from gene targeting experiments, as many of the defects observed in mice that lack a given Hox gene can be attributed to modifications of local growth rates. Homeotic transformations of vertebrae, for example, are often defined as such because of the presence or absence of morphological traits specific for a neighbouring vertebra (e.g. Jeannotte et al., 1993; Kostic and Capecchi, 1994). This can be best explained in terms of either differential recruitment of cells in the cartilage precursors (e.g. by changing adhesive properties of sub-cellular populations), or differential growth and extension of ossification centres within a vertebra, resulting from local changes in the content of HOX proteins (e.g. Kostic and Capecchi, 1994). Loss or reduction of structures (or part of structures) has been observed in the case of internal organs or the hindbrain of mice lacking the activity of a particular Hox gene (Chisaka and Capecchi, 1991; Dollé et al., 1993a). In this respect, the inactivation of the *Hoxd-13* gene, which is the last gene of the HOXD complex (the last and more posteriorly expressed) is revealing. *Hoxd-13*-deficient mice exhibit limbs that seem to have suffered from a developmental arrest. The defect must occur at an early stage of limb development, since a reduction in the extent of the prechondrogenic condensations is observed. This developmental block is subsequently evidenced by a dramatic delay in the ossification of the limbs; the last skeletal elements to be added in normal development will be missing eventually, leading to animals with neotenic limbs (Dollé et al., 1993a; Fig. 2).

ATAVISTIC TRANSFORMATIONS

In *Hoxd-13*-deficient mice, some phenotypic traits are suggestive of atavism; for example, an increase in the number of carpal bones of the distal row (Dollé et al., 1993a). Another example of atavism in Hox-deficient mice is the phenotype observed in mice lacking a functional *Hoxa-2* gene (Rijli et al., 1993; Gendron-Maguire et al., 1993). In these animals, homeotic-like transformations of the skeletal elements derived from the second branchial arch are observed and remodelling of bones in the middle ear has led to the appearance of a bone resembling a pterygoquadrate element, a bone that is found in reptiles (Rijli et al., 1993). Atavistic transformations have also been observed in gain of function experiments in which Hox gene expression was modified using either ubiquitous (Kessel et al., 1990) or more specific promoters, in transgenic mice. A transformation of the occipital bones to vertebra-like structures

(appearance of neural arches) was reported by Lufkin et al. (1992), caused by expression of the *Hoxd-4* gene under the control of the *Hoxa-1* promoter, which was able to drive ectopic expression up to more anterior regions. One could thus imagine that slight modifications to the domains of Hox gene expression may have made important contributions to the evolution of vertebrate morphologies. However, as the overall structure of the Hox gene family seems to be highly conserved between higher and lower vertebrates (F. VanderHoeven and D. D., unpublished data), it is unlikely that many interspecies differences in Hox expression domains derived from loss of Hox genes or significant re-arrangement. Consequently, the approaches described above may not be strictly illustrative of the mechanisms that may have been acting during vertebrate evolution.

POSTERIOR PREVALENCE

One possible way of accommodating these experimental results into a model of how evolution has actually occurred in vertebrates, may be provided by the rule of posterior prevalence. Hox gene inactivation experiments usually lead to phenotypic effects that are restricted to the most anterior part of the expression domain. For example, mice deficient for the *Hoxa-1* gene have no visible alterations in the limbs (Lufkin et al., 1991; Chisaka et al., 1992), even though the gene is expressed in limb buds (Duboule and Dollé, 1989). Similarly, mice lacking the *Hoxb-4* gene show homeotic transformations of most anterior vertebrae only, despite the expression of this gene in more posterior vertebrae (Ramirez-Solis et al., 1993). Thus, the inactivation of a given *Hox* gene generally affects only those structures located at the extreme anterior border of its expression domain, while overexpression seems to induce defects only in those anterior parts of the body where more posterior Hox genes are not expressed. This is in agreement with posterior prevalence, which says that a given Hox gene will exert its function essentially in the domain where this gene is the most posterior of the Hox genes expressed (Duboule, 1991). Most gain of function and loss of function experiments provide some support for the existence of this phenomenon which, to some extent, resembles phenotypic suppression in flies (see Morata et al., 1990; Bachiller et al., 1994). However, as this functional hierarchy must operate exclusively in cells that co-

express Hox genes, the phenotype observed when a given Hox gene is knocked out can be more extensive (ie. derived from more posterior cells) if there is a certain degree of mosaicism in the expression of these genes.

In principle, the result of functional hierarchy among Hox gene products would be to restrict potential phenotypic alterations to a reduced area of the embryo; such restriction may prevent too extensive and deleterious transformations from occurring. In this model, minor changes in the temporal control of Hox gene activation could generate evolutionary transformations of structures very similar in outcome to those reported in the experimental approaches described above. For example, a loss of function mutation would correspond, in phenomenological terms, to a situation in which the expression of a given Hox gene was delayed until a stage when its expression would occur too late to compensate for the retardation, or a stage close to the activation of the next posterior (prevalent) gene. Conversely, a gain of function mutation (causing expression of a posterior gene in more anterior domains) would correspond to an advance in the time of expression of this 'posterior' gene, since Hox transcript domains may be shifted anteriorly if activated too early. It is therefore tempting to propose that loss and gain of function mutations of Hox genes may represent the experimental alternatives to heterochronic variations in temporal colinearity that may have occurred during evolution. Minor modifications in timing may have been at the origin of a wide variety of morphological transformations.

In a genetic system such as the Hox gene network, where parts of the same meristic series (e.g. the vertebrae) are individualized by the progressive temporal expression of a series of physically linked genes, variations of the relative times of activation of these genes are likely to produce structures cor-

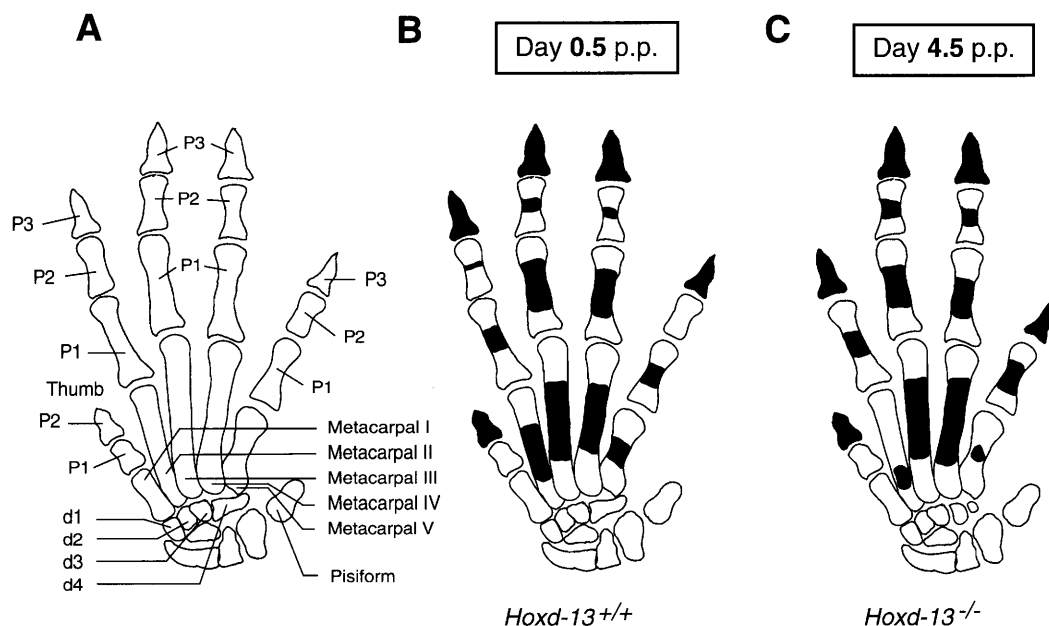


Fig. 2. Illustration of the ossification delay observed in *Hoxd-13*^{-/-} mice. A shows a scheme of a normal hand. The extent of ossification of the various bones is shown in black. B and C show a similar degree of ossification even though the mutated hand (C) is four days older than that of the control littermate (B). This conspicuous delay will result, in adulthood, in hands which will lack some bony elements while the remaining bones will be slightly shorter. In adults, the elements that will be lacking are those that, in normal hands, ossify the last (adapted from Dollé et al., 1993a).

responding to those which are normally produced before or after, within the same series. It is therefore conceivable that heterochronic variations of temporal colinearity make homeotic transformations obligatory rather than exceptional.

A PHYLOTYPIC EGG-TIMER

It is striking that the developmental stage at which Hox genes are sequentially activated corresponds to the so-called phylotypic stage (Sander, 1983): the stage at which vertebrates (for example) express the archetype of the vertebrate body plan (see Wolpert, 1991; Slack et al., 1993, for a discussion and references). This stage (about the early somites stage, but see below) follows the early phase of development and the onset of gastrulation, which, among different species, can be achieved through rather diverse processes, even though molecular analyses suggest a unity of the underlying molecules (e.g. Beddington and Smith, 1993). It precedes the acquisition of species-specific morphological traits which become visible at completion of morphogenesis and organogenesis and thus illustrate that stages of development can exhibit different 'amounts' of evolution (discussed in Raff, 1992). In other words, this stage can be seen as a transition between two phases of development which appear to display some evolutionary variability. Consequently, the phylotypic point, which is (by definition) a stage of high morphogenetic resemblance, can be seen as the narrow point of an ontogenetic egg-timer, a neck of phylogenetic similarity and obligatory passage between two states of higher tolerance for variability (Fig. 3).

In this view, vertebrate embryos, regardless of their early developmental strategies or the way they achieved gastrulation, must converge towards this narrow point to acquire the basic scheme on which subsequent differences will emerge. The fact that Hox genes are expressed at the phylotypic stages of all animal phyla analyzed so far was recently proposed to be a universal trait of animals, hence a basis for the definition of a zootype (Slack et al., 1993). I would like to argue that, in vertebrates, the phylotypic point is neither a point, nor a stage but rather, a *succession* of stages, and propose that the concomitant activation of the Hox gene family is neither a coincidence, nor a consequence of this event, but instead is the *cause* of the apparent invariance of this developmental progression.

There is some difficulty in precisely defining the vertebrate phylotypic stage. It certainly occurs after completion of the major morphogenetic movements, between the head process/early somites

stage and the tail bud stage. At these stages, the rostro-caudal progression of morphogenesis is clearly observable through the sequential condensations of somites from pre-somitic mesoderm and progressive closure of the neural tube. While it is clear that a general inspection of all types of vertebrate embryos reveals the existence of a stage at which they all look globally quite similar to each other, the same degree of similarity can be observed in parts of the same embryos before or after this stage. For example, at the time when rostral structures will start to express some morphological differences amongst classes (e.g. a late somite stage), the gastrulating tail bud will still show similarities in all classes. The definition of a phylotypic point is, therefore, a compromise; the similarity is essentially concerned with a morphogenetic progression, in time, rather than with a punctual state. This may be understood in terms of the obligation, for all vertebrate embryos, to use a developmental mechanism that is intrinsically linked to the temporal sequence corresponding to the developmental stages of the phylotypic progression.

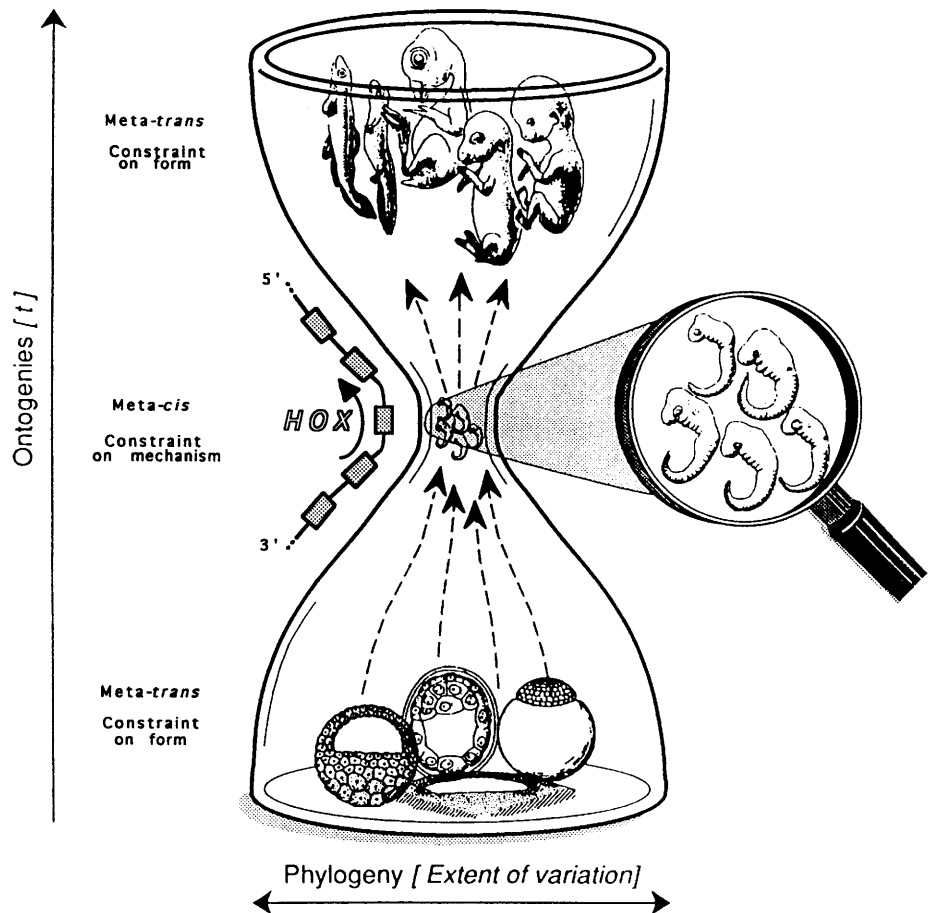


Fig. 3. The phylotypic egg-timer. This scheme illustrates the convergence of vertebrate developmental strategies towards the phylotypic progression, the acquisition of a stable Bauplan. This convergence may be imposed by the necessity to use a meta-cis regulatory mechanism whose intrinsic properties, linked to those of the genetic material, may considerably reduce the amount of evolution allowed within this narrow point (see the text). Such a mechanism may result in the progressive temporal activation of Hox genes along their complexes. The diagrams of embryos are schematic to illustrate the concept; it is not suggested that they give an exact description of vertebrate morphologies.

A META-CIS REGULATORY MECHANISM

Hox genes are turned on sequentially in time and space; for example, in presomitic mesoderm, in parallel with the cranio-caudal morphogenetic progression; this leads to the establishment of a molecular non-equivalence at various levels of the major body axes (e.g. Kessel and Gruss, 1991; Dollé et al., 1989). The pleiotropy of this process (active along all body axes and in many cell types, see above discussion of gene inactivation experiments) and/or a particular mechanistic feature could underlie the observed evolutionary stability of the phylotypic progression. While the pleiotropic effects of Hox gene inactivations are documented (e.g. Dollé et al., 1993a; Small and Potter, 1993), the mechanistic bases of colinearities are as yet unknown. Nevertheless, it appears that the mechanism involved must rely, at least in part, on the physical ordering of genes along segments of DNA, which suggests that the linear structure of our genetic material could be used as a determinant of differences along the linearity of our body axis. The existence of a system of coordinates to encode linear representations of our future body axes within our genetic material itself would explain the invariance of the mechanism involved in the processing of this information. Such a system would be, by essence, invariant as it would rely on the linearity of DNA itself, a feature that may be defined as a meta-cis regulation. This regulation would apply to a supra-genic organization, in contrast to regulatory interactions in *trans* between networks of genes (meta-*trans* regulation); the latter should be prone to a higher evolutionary flexibility (acting upstream and downstream the phylotypic progression).

Possible mechanisms underlying meta-cis regulation (based on the spatial contiguity of genes; discussed by Horder, 1991) are difficult to conceive, but processes involving spreading of chromatin configurations or DNA replication would fit into this category. Such mechanisms impose significant mechanistic constraints on a developmental process and thus make the evolution of morphologies at these developmental stages unlikely; earlier or later stages could accommodate more 'degeneracy', constraints being imposed by the final form rather than by the mechanisms themselves (Edelman, 1988). In vertebrates, the results of such an invariant mechanism underlying colinearities may be primarily observed in anterior or median parts of the body, i.e. those regions where homologies are more easily recognized. In contrast, more posterior regions (in the trunk) or distal regions (in the limbs), under the control of the last Hox genes at the end of the progression, may suffer from both a lack of precision of the mechanism and an increased tolerance for variations and, consequently, be the source of a higher variability. This could account for the 'distal variability versus proximal stability' observed amongst tetrapod limbs (see Hinchliffe, 1991) or for the observation that: "...Nature got tired of counting towards the tail end of a developing animal..." (Goodrich, 1913).

CHANGING SPEED

In vertebrates, not only can the duration of ontogenesis vary considerably, but also the time required for the phylotypic progression. Thus, fishes reach gastrulation well before rodents (in absolute time) and the processing of the fish Hox network

appears to occur faster than in rodents (P. Sordino and D. D., unpublished). This reflects the fact that, whatever mechanism is involved, it can be subject to some variations in its processing speed (heterochronic variations). However, the biochemical nature of a meta-cis regulatory mechanism probably imposes a limit on the extent of possible time compression. It is therefore probable that animals developing faster than the minimum time required for temporal colinearity to proceed had to design different developmental strategies. *Drosophila* may represent an example where the reduction in the time needed to reach the fully segmented germ band stage was too important, and where temporal colinearity has been overridden. Interestingly, in this case the design of an alternative developmental system, plus a corresponding innovation in the segmentation mechanism, correlates with a break of the original HOM complex and the disorganization of ANT-C which, in the context of this model, could be seen as a terminal step in heterochrony (the disappearance of a colinear sequence in the time of activation; Izpisua-Belmonte et al., 1991). As far as the Bithorax complex is concerned, its rather tight organization (which resembles that of the vertebrate HOX complexes; see Duboule, 1992) may simply reflect the fact that most insects develop their abdomen following a time sequence in the addition of segments and may therefore still use temporal colinearity as a mechanism. Thus, it is probable that a correlation exists between a particular mechanism of segmentation (progressive, epimorphic-like), a tight linkage of the corresponding HOM genes within the same complex and the existence of temporal colinearity (a concomitant progressive activation of these genes).

In *Drosophila*, temporal colinearity does not seem to occur anymore, in any part of the body. Accordingly, one might expect the same process that followed the release of this constraint on the ANT-C (the split and disorganization of the complex) should currently be affecting BX-C. In other words, perhaps BX-C can tolerate a degree of disorganization that orthologous pieces of, for example, rodent complexes cannot accept. If this is the case, the differences in organization of the two homeotic complexes in *Drosophila* may simply reflect the fact that an important constraint was released from ANT-C earlier in evolution than its release from BX-C. It is therefore conceivable that the different organization of ANT-C and BX-C reflect two different time points in the evolution of this system in insects. A 'loose' organization (see Duboule, 1992) of the HOM complex is also seen in animals that do not develop according to a segmented body plan, such as *C. elegans* (Bürglin and Ruvkun, 1993), an animal where HOM genes probably specify positions and cell fate but need not be activated in a collinear temporal sequence.

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